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Alterations in SDS-PAGE Profile of Body Muscles of *Cirrhinus mrigala*

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ABSTRACT

The study was carried out with commonly cultured *C. mrigala* exposed to two sub lethal dose levels of 0.025 and 0.05 ppm of arsenic, mercury, nickel and chromium individually and arsenic in combination with mercury, nickel and chromium. Gel electropherograms of muscle protein extracts of *C. mrigala* on their exposure to different heavy metal treatments revealed a definite pattern of variations in their protein profile. It caused synthesis of some additional protein fractions in almost all the treatments with subsequent deletion of some other protein fractions.

KEYWORDS: *Heavy metal toxicity, SDS – PAGE, stress proteins, body muscles, C. mrigala*

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INTRODUCTION

The natural water is one the most precious and vital component of the environment and is essential to all forms of life. The indiscriminate growth of industries particularly dealing with the products like textiles, agro-chemicals, insecticides, tanneries, paints, dye or chloro-alkali metals discharge heavy load of effluents in drainages and rivers. Though, the water bodies have the potential for self-purification, but dumping of wastes exceeds this limit and alters the physiochemical and biological characteristics of water. Development of fisheries in inland water bodies is thus being increasingly impeded due to these environmental constraints, coupled with residual wastes of inorganic fertilizers and pesticides in public health and agricultural sectors (Ghosh *et al.*, 2000).

Today, the heavy metals are termed as 'devil in disguise' and they are kept at top priority list among the water pollutants as they are persistent, water soluble, non-degradable, vigorously oxidizing agents and strongly bind to many biochemical units. They produce cumulative toxicity in small doses over long periods of time and acute toxicity in higher doses. Heavy metals adversely affect various physiological, histological and biochemical functions of fish (Jain and Sharma, 2003; Jain and Mittal, 2004). They exert toxic effects in the organisms at tissue, cellular and molecular level. Toxicities at the cellular level cause disturbances in reproduction, differentiation and maturation as exemplified by teratogenesis. They mainly affect the permeability of the cell membrane and disturb the energy metabolism, and also decrease the stability of lysosomal membrane which disrupts cell functions by releasing various hydrolases at the molecular level. These metals interact with proteins leading to denaturation and precipitation, allosteric effects or enzyme inhibition (Mizrahi and Achituv, 1989). They bind to nucleic acids, leading to irreversible conformational

changes. Heavy metals also induce protein synthesis in fishes (Ali *et al.*, 2003; Boone and Vijayan, 2002). A unique class of proteins called heat shock proteins has been reported to be evolved to resist with the potential for proteins to become unstable or denatured when stressed and thus act as a defense against protein damage. Heat shock proteins are collectively the only molecular mechanisms that animals utilize to tolerate stress. These proteins have pleiotropic effects interacting with multiple systems in diverse ways regulated by the endocrine system. HSPs have critical roles in helping fish cope with environmental change (Wali and Balkhi 2016). This study is thus aimed at highlighting the alterations in protein profile of body muscles of locally cultured fish *C.mrigala* on exposure to sub lethal dose of certain commonly available heavy metal pollutants in water, which would constitute important biomarkers as clinical test for determining heavy metal toxicity in fishes.

MATERIALS AND METHODS

Cirrhinus mrigala weighing 90±15 gram was procured from local fresh water ponds and acclimatized in tank filled with well aerated water for seven days, then treated with As, Hg, Ni and Cr individually and As in combinations with others at 0.025 and 0.05 ppm concentrations in plastic tubs of 40 litre capacity. After 45 days treatment, the fishes were dissected and body muscles of the control and treated fish were analyzed. Samples were prepared by crushing 100 mg of tissue in 1 ml chilled phosphate buffer (0.1 M, pH 7.0) along with 50 mg insoluble PVP using pestle and mortal (rinsed with D.D.W. and dried) under cold conditions. The contents were then centrifuged at 10,000 rpm at 4^0C for 15 min in a refrigerated centrifuge. The supernatant containing the proteins was taken in Eppendorf tubes and pallets were discarded. The supernatant was stored at -20^0C.

Changes in number of proteins were studied for different treatments by SDS-PAGE, using discontinuous buffer system of Laemmli (1970).For molecular weight determination, a mixture of the marker proteins was also electrophoresed simultaneously in the same gel in the wells adjacent to sample wells.

After completion of electrophoresis, staining and background destaining, relative mobilities (Rm values) were calculated for each of the marker proteins and the resolved proteins by the following formula:

$$\text{Rm value} = \frac{\text{Migration distance of protein band (mm)}}{\text{Migration distance of tracking dye (mm)}}$$

R_m value of marker proteins were plotted against log of molecular weights of marker proteins using semi logarithmic paper. Molecular weights of different proteins were estimated by matching their R_m values with appropriate point on the standard curve.

Results and Discussion:

Electropherograms of muscles tissue protein extracts of *C. mrigala* revealed a total of seventeen protein fractions (Table 1, Fig. 1). Two fractions (39.8 and 33.1 kDa) were highly resolved whereas other four protein fractions (151.3, 107.1, 93.3 and 77.6 kDa) were poorly evident over other fractions. On exposure to Hg (0.025) four new fractions of 79.4, 75.8, 45.7 and 29.5 kDa appeared. The protein fraction of 45.7 kDa was of very high concentration while others were poorly expressed. Two more new fractions of 89.1 and 85.1 kDa were added at Hg (0.05 ppm), resulting in a total of twenty-three protein fractions. Expression of a fraction of 26.9 kDa was at higher intensity over the control at both concentration levels. Four similar additional fractions (89.1, 79.4, 75.8 and 45.7 kDa) as in case of Hg (0.05) were also induced due to Ni treatment at 0.025 ppm. Ni at 0.05 ppm also behaved in a similar way, except a fraction of 89.1 kDa. Maximally eleven protein fractions of 112.2, 104.1, 100.0, 97.7, 89.1, 69.1, 50.1, 45.7, 34.2, 31.6 and 21.8 kDa were visible under Cr treatment at 0.025 ppm with subsequent deletion of a highly concentrated fraction of 31.1 kDa and two other fractions of 22.9 and 19.9 kDa over control, resulting in a total of twenty five protein fractions. Cr at 0.05 ppm induced synthesis of seven protein fractions of 104.7, 100.0, 89.1, 72.4, 23.9, 23.4 and 19.9 kDa Mr. Three fractions of 144.5, 117.4 and 190.0 kDa Mr were poorly expressed while 77.6 and 28.1 kDa were highly expressed over control at 0.025 ppm. Intensity of only 144.5 kDa fraction was reduced while of 93.3 and 77.6 kDa fractions was increased over the control at 0.05 ppm level. As (0.025) induced synthesis of two low intensity protein fractions of 89.1 and 72.4 kDa and a highly condense fraction of 45.7 kDa, followed by deletion of 28.1 kDa fraction. As at 0.05 ppm also induced same alterations except addition of a new fraction of

112.2 kDa, followed by deletion of a fraction of 77.6 kDa. Intensity of 93.3 kDa was increased at both concentration levels while of 22.9 kDa at 0.025 ppm and of 144.5 and 117.4 kDa at 0.05 ppm was decreased over control.

When fish was exposed to different combinations of heavy metals (Table 2), electropherograms revealed that Hg in combination with As at both levels induced synthesis of two fraction of 112.2 and 89.1 kDa and deletion of a fraction of 28.1 kDa similar to As (0.05), followed by new addition of 43.6 and 36.3 kDa at both levels and 75.8 kDa at 0.05 ppm only with deletion of a new protein fraction of 26.9 kDa at 0.025 ppm only. Intensity of 117.4, 22.9 and 19.0 kDa fractions was decreased over control at both test concentrations, while of 19.9 kDa was decreased at 0.025 ppm only. Ni in combination with As at 0.025 ppm induced synthesis of five protein fractions of 173.7, 77.6, 75.8, 43.6 and 36.3 kDa, with subsequent deletion of a 28.1 kDa fraction. At 0.05 ppm, 43.6 kDa fraction was not added and a new fraction of 107.1 kDa was deleted. Intensity of 93.3 kDa was increased while of 128.8 and 22.9 kDa was reduced over control at both levels. Intensity of two more fractions 114.5 and 117.4 kDa was also reduced at 0.05 ppm level only. Cr in combination with As induced synthesis of a highly intense protein fraction of 36.3 kDa with five other fraction of 173.7, 100.0, 89.1, 85.1 and 75.8 kDa with subsequent deletion of a 28.1 kDa fraction (deletion was same as that in As treatment). Intensity of 93.3 and 52.4 kDa protein fractions was increase while of 33.1 and 22.9 kDa was decreased over the control.

Proteins are the primary effectors molecules of all living systems and any adaptive responses to environmental, physiological or pathological conditions will be reflected by alterations in protein activity or content (Suneetha et al 2010). The results of the present investigations demonstrated that there were definite qualitative alterations in protein fractions and their protein intensity profiles. In the earlier studies (Suresh *et al*, 1991) heavy metal, mercury was found to inflict cellular metabolism thereby leading impaired protein synthetic machinery in *C. carpio*. The changes in protein profiles recorded after heavy metal treatment were attributed largely to proteotoxic effects of heavy metals. Borgia *et al.* (2019) also reported the appearance or disappearance of protein fractions in the serum of *C. carpio* compared to control fish due to stress caused by metals in the effluent. When damage to proteins occurs, stress proteins commonly called as hsps (Heat shock proteins) are believed to be induced. It has been also known that stress proteins, also known as chaperones, prevent the reactive hydrophobic parts of the damaged proteins from forming non-specific complexes with normal cellular proteins (Weigant *et al.*, 1997). Thus, the alteration in the gel electrophoretic profiles or loss or gain of bands would possibly have some correlations with these stress proteins.

Apart from their protein damaging action, heavy metals have been reported to cause chromosomal damage (Bartoli *et al.*, 1991), increased DNAase activity (Joshi and Desai, 1988), decrease in DNA and RNA levels (Chaudhary, 2004). Therefore, the change of gel electrophoretic band profile might actually reflect damage in DNA or protein synthesizing system by heavy metal treatments.

Various authors (Boone and Vijayan, 2002; Tabche, 2002; Ali *et al.*, 2003; Chaudhary, 2004) reported induction of stress proteins by heavy metals. In this study, it was observed that metals are effective inducers of stress proteins, although the specific stress proteins induced could vary considerably. This was influenced by the type and dose of metal administered. This statement is exactly supported by Goering and Fisher, 1995 and Sanders *et al.*, 1996.This specificity in response makes it difficult to offer generalization regarding the induction of specific stress proteins by metals. These differences in protein induction might also reflect differences in the mechanisms of action by which specific metals elicit toxicity.

Heat shock proteins are the only molecular mechanisms that living organisms adopt to tolerate heavy metal stress, and these proteins have pleiotropic effects, interacting with multiple systems in diverse ways regulated by the endocrine system. Heat shock proteins are important in relation to heavy metal stress resistance and adaptation to the environment. Heat-shock proteins play an important role in regulating a range of effect or components, all of which contribute to survival under heavy metal stress by solving the problem of misfolding and aggregation, as well as its role as chaperones (Joseph et.al.2012).Based on the synthesis of stress proteins on exposure to heavy metals, SDS-PAGE profile

study could constitute important biomarker as clinical test for determining heavy metal toxicity in fishes. Osman et.al. (2010) also inferred protein electrophoresis as a sensitive tool for bio monitoring aquatic pollution.

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Conflict: There is no conflict of interest.

Table 1: Protein profile of muscles of *Cirrhinus mrigala* exposed to different heavy metals

Sr. No.	Rm valve	M.W. (k.Da.)	Control	Hg 0.025	Hg 0.05	Ni 0.025	Ni 0.05	Cr 0.025	Cr 0.05	As 0.025	As 0.05
1	0.009	173.7	-	-	-	-	-	-	-	-	+
2	0.046	151.3	+	+	+	+	+	+	+	+	+
3	0.074	144.5	++	++	++	++	++	+	+	++	+
4	0.12	128.8	++	++	++	++	++	++	++	++	++
5	0.16	117.4	++	++	++	++	++	+	++	++	+
6	0.186	112.2	-	-	-	-	-	+	-	-	+
7	0.205	107.1	+	+	+	+	+	+	+	+	+
8	0.214	104.7	-	-	-	-	-	+	+	-	-
9	0.233	100.0	-	-	-	-	-	+	+	-	-
10	0.242	97.7	-	-	-	-	-	+	-	-	-
11	0.261	93.3	+	++	++	++	++	+	++	++	++
12	0.289	89.1	-	-	+	+	-	++	+	+	+
13	0.308	85.1	-	-	+	-	-	-	-	-	-
14	0.336	79.4	-	+	+	+	+	-	-	-	-
15	0.345	77.6	+	+	+	-	-	++	++	+	-
16	0.353	75.8	-	+	+	+	+	-	-	-	-
17	0.373	72.4	-	-	-	-	-	-	++	+	+
18	0.392	69.1	-	-	-	-	-	++	-	-	-
19	0.429	64.5	++	++	++	++	++	++	++	++	++

Contd… Table 1

Sr. No.	Rm value	M.W. (k.Da.)	Control	Hg 0.025	Hg 0.05	Ni 0.025	Ni 0.05	Cr 0.025	Cr 0.05	As 0.025	As 0.05
20	0.514	52.4	++	++	++	+	+	++	++	++	++
21	0.532	50.1	-	-	-	-	-	++	-	-	-
22	0.579	45.7	-	+++	+++	+	+	+	-	+++	+++
23	0.635	39.8	+++	+++	+++	++	++	+++	+++	+++	++
24	0.700	34.2	-	-	-	-	-	++	-	-	-
25	0.719	33.1	+++	+++	+++	+++	+++	-	++	+++	+++
26	0.747	31.6	-	-	-	-	-	++	-	-	-
27	0.775	29.5	-	+	+	-	-	-	-	-	-
28	0.794	28.1	+	+	+	+	+	+++	+	-	-
29	0.803	26.9	++	+++	+++	++	++	++	++	++	++
30	0.869	23.9	-	-	-	-	-	-	++	-	-
31	0.878	23.4	-	-	-	-	-	-	++	-	-
32	0.887	22.9	++	++	++	++	++	-	-	+	++
33	0.906	21.8	-	-	-	-	-	+++	-	-	-
34	0.934	19.9	++	++	++	++	++	-	++	++	++
35	0.962	19.0	++	++	++	++	++	+	++	++	++
36	0.990	17.7	++	++	++	++	++	++	++	++	++
Total number of proteins			**17**	**21**	**23**	**20**	**19**	**25**	**22**	**19**	**20**

Note -+: Least band intensity; ++: Medium band intensity; +++ Highest band intensity

Table 2: Protein profile of muscles of *Cirrhinus mrigala* exposed to different combination of heavy metals

Sr. No.	Rm value	M.W. (k.Da.)	Control	AS+Hg 0.025	AS+Hg 0.05	AS+Ni 0.025	AS+Ni 0.05	AS+Cr 0.025	AS+Cr 0.05
1	0.009	173.7	-	-	-	+	+	+	+
2	0.046	151.3	+	+	++	+	+	+	+
3	0.074	144.5	++	++	++	++	+	++	++
4	0.12	128.8	++	++	++	+	+	++	++
5	0.16	117.4	++	+	+	++	+	++	++
6	0.18	112.2	-	+	+	-	-	-	-
7	0.20	107.1	+	+	+	+	-	+	+
8	0.23	100.0	-	-	-	-	-	+	+
9	0.26	93.3	+	+	++	++	++	++	++
10	0.28	89.1	-	+	+	-	-	+	+
11	0.30	85.1	-	-	-	-	-	+	+
12	0.34	77.6	+	+	+	+	+	+	+
13	0.35	75.8	-	-	+	+	+	+	+

Contd… Table 2

Sr. No.	Rm value	M.W. (k.Da.)	Control	AS+Hg 0.025	AS+Hg 0.05	AS+Ni 0.025	AS+Ni 0.05	AS+Cr 0.025	AS+Cr 0.05
14	0.42	64.5	++	++	++	++	++	++	++
15	0.51	52.4	++	+	++	++	++	+++	+++
16	0.59	43.6	-	+	+++	++	-	-	-
17	0.63	39.8	+++	++	+++	+++	+++	+++	+++
18	0.67	36.3	-	+	++	+	+	+++	+++
19	0.71	33.1	+++	+	+++	+++	+++	++	++
20	0.79	28.1	+	-	-	-	-	-	-
21	0.80	26.9	++	-	++	++	++	++	++
22	0.88	22.9	++	+	+	+	+	+	+
23	0.93	19.9	++	+	++	++	++	++	++
24	0.96	19.0	++	+	+	++	++	++	++
25	0.99	17.7	++	+	+	++	++	++	++
Total number of proteins			**17**	**19**	**21**	**20**	**18**	**22**	**22**

Note -+: Least band intensity; ++: Medium band intensity; +++ Highest band intensity

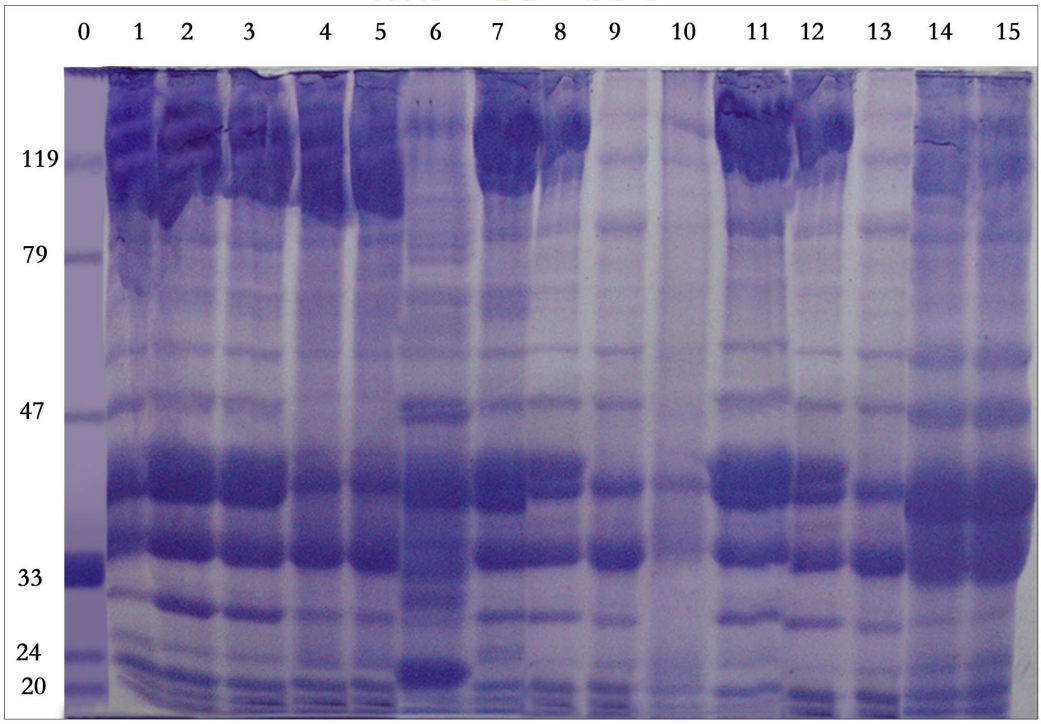

Fig. 1: Gel electropherogram of body muscles of *C. mrigala* exposed to different heavy metal treatments

Lane 0- Molecular weight marker (molecular weight (kDa) of each band is given at side).

Lane 1-Control; Lane 2 - Hg (0.025); Lane 3 - Hg (0.05)

Lane 4- Ni (0.025); Lane 5 - Ni (0.05); Lane 6 - Cr (0.025)

Lane 7- Cr (0.05); Lane 8 - As (0.025); Lane 9- As (0.05)

Lane 10 -As + Hg (0.025)' Lane 11 - As + Hg (0.05)

Lane 12 -As + Ni (0.025); Lane 13 - As + Ni (0.05)

Lane 14 -As + Cr (0.025); Lane 15 - As + Cr (0.05)

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